Electron beam and gamma irradiation as feasible conservation technologies for wild *Arenaria montana* L.: effects on chemical and antioxidant parameters

Running Title: Electron beam and gamma irradiation of *Arenaria montana*

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ABSTRACT

Wild plants are widely recognized as high-potential sources of several bioactive compounds. Nevertheless, these natural matrices require effective decontamination steps before they might be considered for different industrial purposes. Irradiation techniques are being progressively acknowledged as feasible conservation methodologies, either for their high decontamination effectiveness, as well as for their innocuousness on most chemical and bioactive parameters of the material to be treated. *Arenaria montana* L. (Caryophyllaceae) is recognized for its phytochemical richness, having a relevant geographical distribution in the Southern Europe. Herein the effects of irradiation (gamma and electron beam up to 10 kGy) were evaluated by comparing the nutritional, chemical and antioxidant profiles in *A. montana* extracts. In general, the assayed parameters showed statistically significant variations in response to irradiation treatment. Furthermore, the performed LDA allowed identifying the antioxidant indicators as the most affected parameters in irradiated samples, especially when using the 10 kGy dose and e-beam irradiation.

Keywords: Gamma irradiation; electron beam irradiation; *Arenaria montana* L.; chemical composition; antioxidant activity.

1. Introduction

Traditional medicine is world-widely used for several disorders and is based on natural products with specific physiological actions on the human body (Adebayo, Dzoyem, Shai, & Eloff, 2015; Ibrahim, Mohammed, Isah, & Aliyu, 2014). The rich composition of plants in tocopherols, alkaloids, tannins, flavonoids and other phenolic compounds, terpenoids and saponins makes them effective and beneficial on lipid metabolism, stimulating digestion, acting as anti-diabetics, as also as antioxidant and anti-inflammatory agents (Skotti, Anastasaki, Kanellou, Polissiou, & Tarantilis, 2014; Rawat, Bhatt, & Rawal 2011).

The growing demands for natural sources of bioactive compounds have stimulated various studies with the purpose of discover new pharmacological compounds with lower toxicity (Haleem, Salem, Fatahallah, & Abdelfattah 2014; Lubbe & Verpoorte, 2011). However, the fact that the pharmaceutical industry is very strict regarding microbiological quality of raw materials makes necessary the application of decontamination techniques (Haleem, Salem, Fatahallah, & Abdelfattah 2014; Katušin-Ražem, Novak, & Ražema, 2001).

Irradiation is a methodology accredited for dry ingredients and can be performed using various radiation sources (*e.g.*, gamma rays, electron beam and X-rays) and doses, in accordance with the objectives to be achieved. This technique is increasingly recognized throughout the world and is characterized as eliminating or being reducing microorganisms, parasites and pests without causing any change (chemical or organoleptic) in food, being safe for the consumer and also allowing a reduction of the use of chemical fumigants (Jung et al., 2015; Owczarczyk, Migdal, & Kędzia 2000; Roberts, 2014; Shim et al., 2009; Supriya, Sridhar, & Ganesh, 2014; Van Calenberg et al., 1998).

Electron beam irradiation is used mainly for food products with low density and the equipment can be easily connected/disconnected. Otherwise, gamma irradiation is mainly used for large volumes (Fernandes et al., 2014; Van Calenberg et al., 1998).

Arenaria montana L. is a flowering plant belonging to the Caryophyllaceae family, native to mountainous regions of southwestern Europe. It is used in the Portuguese traditional medicine, acting therapeutically as an anti-inflammatory and diuretic, being mainly ingested in the form of infusion, prepared from the leaves, stems and flowers (Timité et al., 2011; Carvalho, & Morales, 2013). Moreover, we previously described its antioxidant potential and richness in bioactive phytochemicals (Pereira et al., 2014).

In the present study, the objective was to evaluate the effects of irradiation (gamma and electron beam) at different doses (1 and 10 kGy) in nutritional, chemical and antioxidant parameters of *A. montana*.

2. Materials and methods

2.1. Samples and samples irradiation

Arenaria montana L. (Caryophyllaceae) flowers and leafy stems (approximately the upper 15 cm of the dense clumps produced in Spring) are commonly gathered in the wild regions of Northeastern Portugal. These plant materials are usually dried and kept in dark, to be prepared in infusion and used as homemade remedies. Considering the availability and local consumers' criteria for its medicinal use, *A. montana* was collected in full bloom, in Spring along paths through the oak forest, in Oleiros, Bragança, Portugal. The sample for analysis was made by putting together the vegetative material from different plants randomly selected. A voucher specimen was deposited at the Herbarium of the of the School of Agriculture of Bragança, Portugal.

Then, the sampled material was divided for gamma and for e-beam irradiation, in control (non-irradiated, 0 kGy), group 1 (1 kGy) and group 2 (10 kGy).

Gamma irradiation: The irradiation was performed in a Co-60 experimental chamber (Precisa 22, Graviner Manufacturing Company Ltd., UK) with total activity 177 TBq (4.78 kCi), in September 2013, and the estimated dose rate for the irradiation position was obtained with the Fricke dosimeter. During irradiation process, the dose was estimated using Amber Perspex routine dosimeters (batch V, from Harwell Company, U.K.), following the procedure previously described by Pereira et al. (2015). The estimated doses were, respectively: 0.92 ± 0.01 kGy, 1.9 kGy h^{-1}, 1.1 for sample 1 and 8.97 ± 0.35 kGy, , 1.2 for sample 2, both at a dose rate of 1.9 kGy $h^{-1}$and 1.2 dose uniformity ratio (D_{max}/D_{min}). For simplicity, in the text and tables we considered the values 0, 1 and 10 kGy, for the doses of non-irradiated and irradiated groups 1 and 2, respectively.

Electron beam irradiation: The irradiation was performed at the INCT- Institute of Nuclear Chemistry and Technology, in Warsaw, Poland. To estimate the dose during the irradiation process three types of dosimeters were used: a standard dosimeter, a graphite calorimeter, and two routine Gammachrome YR and Amber Perspex dosimeters, from Harwell Company (UK). The irradiation took place in an e-beam irradiator of 10 MeV of energy with pulse duration of 5.5 ms, pulse frequency of 440 Hz and average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was settled to the range 20-100 cm/min and the scan frequency was 5 Hz. The absorbed dose for e-beam irradiated *A. montana* were, 0.83 and 10.09 kGy, for group 1 and group 2 respectively, measured with a maximum uncertainty of 20%. To read the Amber and

Gammachrome YR dosimeters, spectrophotometric methods were used at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previous calibration curve. For the graphite calorimeter dosimeter the electrical resistance was read and converted in dose according to a calibrated curve, available at the facility and made during equipment routine calibrations.

2.2. Standards and reagents

For irradiation: To estimate the dose and dose rate for gamma irradiation it was used a chemical solution sensitive to ionizing radiation, Fricke dosimeter, prepared in the lab following the standards (ASTM, 1992) and during irradiations Amber Perspex routine dosimeters (batch V, from Harwell Dosimeters Ltd, Oxfordshire, UK) were used, previously calibrated against the standard dosimeter. To prepare the acid aqueous Fricke dosimeter solution the following reagents were used: ferrous ammonium sulphate(II) hexahydrate, sodium chloride and sulfuric acid, all purchased from Panreac S.A. (Barcelona, Spain) with purity PA (proanalysis), and water treated in a Milli-Q water purification system (Millipore, model A10, USA). For e-beam routine irradiation were used Gammachrome YR and Amber Perspex routine dosimeters (batch V, from Harwell Dosimeters Ltd, Oxfordshire, UK) and a graphite calorimeter as standard dosimeter.

For chemical analyses: Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (Lisbon, Portugal). Fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA), as also were other individual fatty acid isomers, L-ascorbic acid, tocopherol, sugar and organic acid standards, and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Racemic tocol, 50 mg/mL, was

purchased from Matreya (Pleasant Gap, PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA).

2.3. Nutritional value

Protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC 1995). The samples crude protein content (N×6.25) was estimated by the macro-Kjeldahl method; the crude fat was determined using a Soxhlet apparatus by extracting a known weight of sample with petroleum ether; the ash content was determined by incineration at 600±15 °C, until a whitish ash was formed. Total carbohydrates were calculated by difference and total energy was calculated according to the following equations: Energy (kcal)=4×(g protein+g carbohydrates)+9×(g fat).

2.4. Phytochemical composition in hydrophilic compounds

Sugars. Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI), after an extraction procedure previously described by the authors (Pereira et al., 2015) using melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco, Easton, MD, USA) and a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards.

Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

Organic acids. Organic acids were determined following a procedure previously described by the authors (Pereira et al., 2015). The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Cooperation, Kyoto, Japan). Separation was achieved on a SphereClone (Phenomenex, Torrance, CA, USA) reverse phase C_{18} column (5 µm, 250 mm × 4.6 mm i.d) thermostatted at 35 ºC. The elution was performed with sulphuric acid 3.6 mM using a flow rate of 0.8 mL/min. Detection was carried out in a DAD, using 215 nm and 245 nm (for ascorbic acid) as preferred wavelengths. The organic acids found were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g dw.

2.5. Phytochemical composition in lipophilic compounds

Fatty acids. Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Pereira et al., 2015). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID at 260 ºC) and a Macherey-Nagel (Duren, Germany) column (50% cyanopropyl-methyl-50% phenylmethylpolysiloxane, 30 m × 0.32 mm ID × 0.25 µm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50

ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using the CSW 1.7 Software (DataApex, Prague, Czech Republic) and expressed in relative percentage of each fatty acid.

Tocopherols. Tocopherols were determined following a procedure previously described by the authors (Pereira et al., 2015). Analysis was performed by HPLC (equipment described above), and a fluorescence detector (model FP-2020, Jasco International Co., Tokyo, Japan) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II (250 × 4.6 mm) normal-phase column (model YMC, Waters Corporation, Milford Massachusetts, USA) operating at 30 ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min, and the injection volume was 20 µL. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the Internal Standard (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight.

2.6. Evaluation of antioxidant activity

Samples preparation.

Two different extracts were prepared to evaluate their antioxidant activity.

The methanolic extract was obtained from the dried plant material. The sample (1 g) was extracted by stirring with 25 mL of methanol (25 ºC at 150 rpm) for 1 h and subsequently filtered through Whatman No. 4 paper (Sigma-Aldrich, St. Louis,

Missouri, USA). The residue was then extracted with 25 mL of methanol (25 ºC at 150 rpm) for 1 h. The combined methanolic extracts were evaporated at 40 ºC (rotary evaporator Büchi R-210, Flawil, Switzerland) to dryness.

The infusion was also obtained from the dried plant material. The sample (2 g) was added to 200 mL of boiling distilled water and left to stand at room temperature for 5 min, and then filtered under reduced pressure.

Antioxidant activity. DPPH radical-scavenging activity was evaluated by using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, VT, USA), and calculated as a percentage of DPPH discolouration using the formula: $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution. Reducing power was evaluated by the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching was evaluated though the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which is measured by the formula: β-carotene absorbance after 2h of assay/initial absorbance) × 100. Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decreasing in thiobarbituric acid reactive substances (TBARS); the colour intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the following formula: [(A - B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively (Pereira et al., 2015).

2.8. Statistical analysis

Within each irradiation type and for each dose, three independent samples were analysed. Each of the samples was taken after pooling the plants treated in the same conditions together. Data were expressed as mean±standard deviation. All statistical tests were performed at a 5% significance level using IBM SPSS Statistics for Windows, version 22.0. (IBM Corp., USA).

An analysis of variance (ANOVA), followed by Tukey's test (homoscedastic distributions) or Tamhane's T2 test (heteroscedastic distributions) was used to classify the statistical differences induced by the irradiation dose in each of the assayed parameters. The fulfillment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively.

Linear discriminant analysis (LDA) was used to identify the parameters undergoing the most significant changes for i) each irradiation dose and ii) each irradiation type. A stepwise technique was applied, based on the Wilks' Λ test with the usual probabilities of F (3.84 to enter and 2.71 to be removed) for variable selection. This procedure combines a series of forward selection and backward elimination steps, where the inclusion of any new variable is preceded by verifying the significance of all previously selected variables (Zielinsky et al., 2014). In the present study, the purposes of the performed LDA were identifying the relationship between a single categorical dependent variable (irradiation dose or irradiation type) and the set of quantitative independent variables (studied parameters). With this method, it is possible to determine which of the independent variables contributed more for the differences in the average score profiles of *A. montana* samples submitted to each irradiation type and dose. To verify the significance of the canonical discriminating functions, Wilk's Λ test was used.

A leaving-one-out cross validation procedure was carried out to assess the model performance.

3. Results and discussion

As previous consideration, it should be highlighted that there were no available reports (at the moment of preparation of this manuscript) on the chemical composition or antioxidant activity of *A. montana*, except for our work on samples gathered in a different year (Pereira et al., 2014), which described the cytotoxicity and phenolic composition of this species, but in non-irradiated samples.

3.1. Effects on chemical parameters

The proximate composition (in dry basis) of *A. montana* was dominated by the carbohydrates content, followed by ash, protein and fat levels (**Table 1**). Except for the fat content in gamma irradiated samples (p = 0.051), all the nutritional components showed significant changes in response to irradiation treatment, either for electron beam (e-beam), as well as in gamma irradiated samples. In the first case (e-beam), fat and protein contents decreased in irradiated samples, while ash, carbohydrates and energy values only showed slight variations. The effect produced by gamma irradiation on the nutritional parameters was similar to the one observed for electron beam irradiation, except for the already pointed out lack of variation in fat content and the less pronounced decrease observed in proteins.

Regarding the free sugars composition (**Table 2**), fructose was quantified as the main compound (4.2-4.7 g/100 g dw), followed by glucose (1.5-1.7 g/100 g dw), sucrose (0.4-1.0 g/100 g dw), trehalose (0.19-0.23 g/100 g dw) and raffinose (0.09-0.13 g/100 g dw). The detected profile and individual proportions are similar to those presented in a

previous report (Pereira et al., 2014), despite the overall quantities detected in this work are slightly lower. A good result was obtained for the effect of irradiation treatment, since almost none of the characterized molecules suffer significant changes (except for sucrose with both irradiation types and total sugars when samples were treated with e-beam irradiation). This is particularly valuable because sugars are often pointed out as good indicators of an adequate conservation technology (Barreira, Pereira, Oliveira, & Ferreira, 2010).

Concerning the organic acids profile, oxalic acid was the prevalent form (2.2-2.6 g/100 g dw), followed by malic acid (0.9-1.1 g/100 g dw), succinic acid (0.5-0.7 g/100 g dw), citric acid (0.29-0.38 g/100 g dw), quinic acid and fumaric acid (which were detected below the limit of quantification); this result is also closely related to the mentioned previous report (Pereira et al., 2014). The dissimilarity observed among the effect produced by each type of irradiation is quite interesting. In fact, while e-beam irradiation did not cause statistically significant changes in any case, gamma irradiation produced exactly the opposite effect, *i.e.*, all the quantified organic acids presented significant changes, with a clear tendency to increase with irradiation. This result is in agreement with a previous study conducted to evaluate the effects of gamma irradiation and accelerated electrons on organic acids (Semelová, Čuba, John, & Múčka 2008).

The individual fatty acids (FA) profiles are depicted in **Table 4**. Besides the presented FA, C6:0, C8:0, C10:0, C13:0, C16:1, C20:2, C20:3n3+C21:0 and C23:0 were also detected, but their relative percentages laid below 0.5%. In general, the detected profile is highly similar to the one reported before (Pereira et al., 2014).

The main saturated fatty acid (SFA) was palmitic acid (22-26%), while oleic acid (10.1-13.4%) and α-linolenic acid (17.4-22.7%) were the predominant monounsaturated and polyunsaturated forms, respectively. Nearly half of the detected forms are

polyunsaturated fatty acids (PUFA), among which ALA (α-linolenic acid) and GLA (γ-linolenic acid) deserve special attention. Despite the significant changes induced by both irradiation types in all FA, the effect of e-beam treatment was more pronounced. Considering that the results are presented in relative percentage, if some FA decrease significantly, others will, inevitably, increase. Nevertheless, when evaluated as grouped SFA, MUFA and PUFA, it became clear that e-beam treatment increased the relative percentages of SFA and MUFA, while reducing those of PUFA.

The tocopherol profile was similar to the previously characterized (Pereira et al., 2014), despite the higher quantities detected herein. α-Tocopherol was the main isoform, showing nearly 20-fold higher amounts than the remaining vitamers. In what concerns the main subject of this work, the irradiation treatment caused statistically significant changes in the tocopherols contents, especially in samples irradiated with 10 kGy. The significant effect of irradiation over the tocopherols content was previously reported (Pereira et al., 2015; Taipina, Lamardo, Rodas, & Mastro, 2009), being probably associated to their oxidative instability (Warner, Miller, & Demurin, 2008).

3.2. Effects on the antioxidant activity

Besides comparing the chemical parameters described in the previous section, the effects induced by gamma and e-beam irradiation on the antioxidant activity of *A. montana* were also compared in its aqueous and methanolic extracts. Four *in vitro* assays were applied: scavenging effects on DPPH radicals (measures the decrease in DPPH radical absorption after exposure to radical scavengers), reducing power (conversion of a Fe^{3+}/ferricyanide complex to Fe^{2+}), inhibition of β-carotene bleaching (measures the capacity to neutralize the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models) and

thiobarbituric acid reactive substances (TBARS) formation inhibition. Likewise, an overall quantification of total phenols was also performed (**Table 5**). The methanolic extracts showed higher (approximately threefold) antioxidant activity than the corresponding infusions in all performed assays. In agreement with these results, the quantities of phenolic compounds were nearly threefold lower in the infusions. Furthermore, *A. montana* extracts were particularly active as inhibitors of TBARS formation, as indicated by the lowest EC_{50} values detected in this case.

Independently of irradiation type, the antioxidant activity decreased in the infusions along the irradiation treatment, in line with the observed among the methanolic extracts when treated with gamma irradiation. Nevertheless, the effect observed in the methanolic extracts submitted to e-beam irradiation showed a general tendency to increased antioxidant activity, except for the β-carotene bleaching inhibition.

3.3. Linear discriminant analysis

As indicated by the results presented in **Tables 1-5**, most of the characterized parameters (with the exceptions of the majority of free sugars and organic acids) showed statistically significant differences when submitted to gamma or e-beam irradiation treatment. To take this comparative study a step further, the overall significance of the detected differences was also evaluated by verifying which statistically significant differences maintain their relevance when compared globally. Accordingly, the results were evaluated simultaneously by applying two sequential LDA: initially the results were grouped according to irradiation type, while in the second case the grouping criterion was based on the irradiation dose. The significant independent variables were selected using the stepwise procedure of the LDA,

according to the Wilks' λ test, which maintains only those with a statistically significant ($p < 0.05$) classification ability.

The two discriminant functions plotted in **Figure 1A**, included 100.0% of the observed variance (first: 80.0%, second: 20.0%). As an initial result, the reduction in the variables number was noteworthy. From the initial 58 parameters, only 11 (fat, carbohydrates, raffinose, C6:0, C20:4n6, C20:5n3, C24:0, reducing power in infusions, TBARS formation inhibition and β-carotene bleaching inhibition in methanolic extracts, phenols content in infusions) were selected as having discriminant ability. Concerning the correlation between the selected discriminating variables and the canonical discriminant functions, function 1 was more correlated with TBARS formation inhibition in methanolic extracts (which present higher EC_{50} values in gamma irradiated samples) and fat (higher values in gamma irradiated samples), separating mostly gamma irradiated samples from the remaining groups. Function 2, in turn, was more correlated to the reducing power (lower in e-beam irradiated samples) and phenolic content in the *A. montana* infusions (lower in e-beam irradiated samples), clearly separating the markers corresponding to e-beam irradiated samples from those belonging to unirradiated ones. The classification performance was 100% accurate, either for the originally grouped cases, as well as for the cross-validated cases.

When a similar assay was conducted to evaluate the variables undergoing the most relevant changes once submitted to different irradiation doses (independently of being generated by gamma rays or accelerated electrons), the two discriminant functions (**Figure 1B**) also included 100.0% of the observed variance (first: 78.8%, second: 20.2%).

The reduction in the variables number was significant again, indicating carbohydrates, C6:0, C8:0, C20:4n6, C20:5n3, SFA, γ-tocopherol, reducing power EC_{50} values in

infusions, DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and TBARS formation inhibition in methanolic extracts and phenols content in the infusions as the variables with the highest changes. Function 1 was highly correlated with reducing power (which present higher EC_{50} values for the 10 kGy dose) and phenolic content (lower in samples irradiated with 10 kGy), clearly separating the markers corresponding to the 10 kGy dose. Function 2, on the other hand, was more correlated to C6:0 (higher in samples irradiated with 1 kGy) and SFA (lower in samples with 1 kGy), particularly contributing to separate the markers corresponding to samples irradiated with 1 kGy (independently of irradiation source). The classification performance was 100% accurate, either for the originally grouped cases, as well as for the cross-validated cases.

4. Conclusions

Most of the assayed parameters (except for the majority of sugars and organic acids) showed statistically significant variations in response to irradiation treatment. Nevertheless, the performed LDA allowed defining which of the studied parameters were mostly affected by gamma or e-beam irradiation, as well as by using 1 or 10 kGy. In fact, the antioxidant parameters proved to be the ones suffering the most significant changes, especially when using the 10 kGy dose and e-beam irradiation. In general, the obtained results might be a good guidance to choose irradiation type or dose according to the need of maintain a specific chemical or bioactive profile.

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References

Adebayo, S.A., Dzoyem, J.P., Shai L.J., & Eloff, J.N. (2015). The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. *BMC Complementary and Alternative Medicine*, *15*, 159, DOI 10.1186/s12906-015-0669-5.

AOAC (1995). Official Methods of Analysis, vol. 16. Association of Official Analytical Chemists, Arlington VA, USA.

ASTM, American Society for Testing and Materials (1992). Practice for Using the Fricke Reference Standard Dosimetry System, ASTM E1026. Annual Book of ASTM Standards, 12.02, Philadelphia, PA.

Barreira, J.C.M., Pereira, J.A., Oliveira, M.B.P.P., & Ferreira, I.C.F.R. (2010). Sugars profiles of different chestnut (*Castanea sativa* Mill.) and Almond (*Prunus dulcis*) cultivars by HPLC-RI. *Plant Foods for Human Nutrition, 65*, 38-43.

Carvalho A. M., & Morales, R. in Ethnobotany in the New Europe: People, ed. M. Pardo de Santayana, A. Pieroni and R. Puri, Berghahn Books, Oxford, UK, 2nd ed, 2013, pp. 147-171.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2014). Combined effects of electron-beam irradiation and

storage time on the chemical and antioxidant parameters of wild *Macrolepiota procera* dried samples. *Food and Bioprocess Technology, 7*, 1606-1617.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Morales, P., Férnandez-Ruiz, V., Martins, A., Oliveira, M.B.P.P., & Ferreira, I.C.F.R. (2015). Exquisite wild mushrooms as a source of dietary fiber: Analysis in electron-beam irradiated samples. *LWT - Food Science and Technology, 60*, 855-859.

Haleem, R.M., Salem, M.Y., Fatahallah, F.A., & Abdelfattah L.E. (2014). Quality in the pharmaceutical industry - A literature review. *Saudi Pharmaceutical Journal*, dx.doi.org/10.1016/j.jsps.2013.11.004.

Ibrahim, M.A., Mohammed, A., Isah, M.B., & Aliyu, A.B. (2014). Anti-trypanosomal activity of African medicinal plants: A review update. *Journal of Ethnopharmacology, 154*, 26-54.

Jung, K., Song, B-S., Kim, M.J., Moon, B-G., Go, S-M., Kim, J-K., Lee, Y-J., & Park J-H. 2015. Effect of X-ray, gamma ray, and electron beam irradiation on the hygienic and physicochemical qualities of red pepper powder. *LWT - Food Science and Technology, 63*, 846-851.

Katušin-Ražema, B., Novak, B., & Ražema, D. (2001). Microbiological decontamination of botanical raw materials and corresponding pharmaceutical products by irradiation. *Radiation Physics and Chemistry, 62*, 261-275.

Lubbe, A., & Verpoorte, R. (2011). Cultivation of medicinal and aromatic plants for specialty industrial materials. *Industrial Crops and Products, 34*, 785-801.

Owczarczyka, H.B., Migdal, W., & B. Kędzia. (2000). The pharmacological activity of medical herbs after microbiological decontamination by irradiation. *Radiation Physics and Chemistry, 57*, 331-335.

Pereira, E., Barros, L., Calhelha, R.C., Dueñas, M., Carvalho, A.M., Santos-Buelga, C., & Ferreira, I.C.F.R. (2014). Bioactivity and phytochemical characterization of *Arenaria montana* L.. *Food & Function, 5*, 1848.

Pereira, E., Antonio, A.L., Barreira, J.C.M., Barros, L., Bento, A., & Ferreira, I.C.F.R. (2015). Gamma irradiation as a practical alternative to preserve the chemical and bioactive wholesomeness of widely used aromatic plants. *Food Research International, 67*, 338-348.

Rawat, S., Bhatt, I.D., & Rawal, R.S. (2011). Total phenolic compounds and antioxidant potential of *Hedychium spicatum* Buch. Ham. ex D. Don in west Himalaya, India. *Journal of Food Composition and Analysis, 24*, 574–579.

Roberts, P.B. (2014). Food irradiation is safe: Half a century of studies. *Radiation Physics and Chemistry, 105*, 78-82.

Semelová, M., Čuba, V., John, J., & Múčka, V. (2008). Radiolysis of oxalic and citric acids using gamma rays and accelerated electrons. *Radiation Physics and Chemistry*, *77,* 884-888.

Shim, S-L., Hwang, I-M., Ryu, K-Y., Jung, M-S., Seo, H-Y., Kim, H-Y., Song, H-P., Kim, J-H., Lee, J-W., Byun, M-W., Kwon, J-H., & Kim K-S. (2009). Effect of γ-irradiation on the volatile compounds of medicinal herb, Paeoniae Radix. *Radiation Physics and Chemistry, 78*, 665-669.

Skotti, E., Anastasaki, E., Kanellou, G., Polissiou, M., & Tarantilis, P.A. (2014). Total phenolic content, antioxidant activity and toxicity of aqueous extracts from selected Greek medicinal and aromatic plants. Industrial Crops and Products, 53, 46-54.

Supriya, P., Sridhar, K.R., & Ganesh, S. (2014). Fungal decontamination and enhancement of shelf life of edible split beans of wild legume Canavalia maritima by the electron beam irradiation. *Radiation Physics and Chemistry, 96*, 5-11.

Taipina, M.S., Lamardo, L.C.A., Rodas, M.A.B., & Mastro, N.L. (2009). The effects of gamma irradiation on the vitamin E content and sensory qualities of pecan nuts (*Carya illinoensis*). *Radiation Physics and Chemistry, 78*, 611-613.

Timité, G., Mitaine-Offer, A-C., Miyamoto, T., Tanaka, C., Mirjolet, J-F., Duchamp, O., & Lacaille-Dubois, M-A. (2011). Unusual oleanane-type saponins from *Arenaria montana*. Phytochemistry, 72, 503-507.

Van Calenberg, S., Vanhaelewyn, G., Van Cleemput, O., Callens, F., Mondelaers, W., & Huyghebaert, A. (1998). Comparison of the Effect of X-ray and Electron Beam Irradiation on Some Selected Spices. *Lebensmittel-Wissenschaft und-Technologie, 31*, 252-258.

Warner, K., Miller, J., & Demurin, Y. (2008). Oxidative stability of crude mid-oleic sunflower oils from seeds with γ- and δ-tocopherol levels. *Journal of the American Chemical Society, 85*, 529-533.

Zielinski, A.A.F., Haminiuk, C.W.I., Alberti, A., Nogueira, A., Demiate, I.M., & Granato, D. (2014). A comparative study of the phenolic compounds and the in vitro antioxidant activity of different Brazilian teas using multivariate statistical techniques. *Food Research International*, *60*, 246-254.

Figure 1. Canonical discriminant functions coefficients defined from the evaluated parameters plotted to highlight differences among irradiation technologies (**A**) and irradiation doses (**B**).

Table 1. Proximate composition of *Arenaria montana* submitted do different doses of electron-beam or gamma irradiation.[1]

		Fat (g/100 g fw)	Protein (g/100 g dw)	Ash (g/100 g dw)	Carbohydrates (g/100 g dw)	Energy (kcal/100 g dw)
E-beam irradiation						
Dose	0 kGy	1.4±0.1[a]	4.9±0.3[a]	8.4±0.3[ab]	85.4±0.3[b]	373±1[a]
	1 kGy	1.2±0.1[b]	3.9±0.3[b]	8.2±0.2[b]	86.7±0.4[a]	373±1[a]
	10 kGy	1.2±0.1[b]	3.6±0.2[c]	8.6±0.2[a]	86.7±0.4[a]	372±1[b]
p-values	Homoscedasticity[2]	0.451	0.891	0.111	0.231	0.058
	Normal distribution[3]	0.373	0.080	0.346	0.102	0.794
	1-way ANOVA[4]	<0.001	<0.001	0.007	<0.001	0.004
Gamma irradiation						
Dose	0 kGy	1.7±0.1	4.4±0.5[b]	9.6±0.2[a]	84.3±0.5[b]	370±1[b]
	1 kGy	1.7±0.1	5.1±0.3[a]	9.1±0.2[b]	84.1±0.3[b]	372±1[a]
	10 kGy	1.6±0.1	3.8±0.2[c]	9.5±0.2[a]	85.1±0.2[a]	370±1[b]
p-values	Homoscedasticity[2]	0.824	0.011	0.851	0.004	0.760
	Normal distribution[3]	0.448	0.020	0.621	0.106	0.148
	1-way ANOVA[4]	0.051	<0.001	<0.001	<0.001	<0.001

[1]The results are presented as the mean±SD. [2]Homoscedasticity among irradiation doses was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test. [4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one irradiation dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

Table 2. Hydrophilic compounds (free sugars and organic acids) composition (g/100 g dw) of *Arenaria montana* submitted do different doses of electron-beam or gamma irradiation.[1]

		Free sugars						Oxalic acids				
		Fructose	Glucose	Sucrose	Trehalose	Raffinose	Total	Oxalic acid	Malic acid	Citric acid	Succinic acid	Total
E-beam irradiation												
Dose	0 kGy	4.5±0.3	1.6±0.1	0.6±0.1^{b}	0.22±0.03	0.11±0.02	7.1±0.3^{ab}	2.3±0.1	0.9±0.1	0.30±0.05	0.5±0.1	4.0±0.3
	1 kGy	4.2±0.3	1.5±0.1	0.9±0.1^{a}	0.19±0.02	0.12±0.01	6.9±0.4^{b}	2.2±0.2	0.9±0.1	0.29±0.04	0.5±0.1	3.8±0.2
	10 kGy	4.4±0.4	1.7±0.2	1.0±0.1^{a}	0.21±0.04	0.13±0.02	7.3±0.3^{a}	2.2±0.2	0.9±0.1	0.31±0.05	0.5±0.1	3.9±0.3
p-values	Homoscedasticity[2]	0.435	0.462	0.119	0.221	0.842	0.560	0.579	0.669	0.795	0.072	0.737
	Normal distribution[3]	0.123	0.712	0.150	0.206	0.818	0.390	0.762	0.688	0.360	0.377	0.852
	1-way ANOVA[4]	0.195	0.135	<0.001	0.217	0.082	0.034	0.182	0.369	0.743	0.988	0.278
Gamma irradiation												
Dose	0 kGy	4.7±0.3	1.7±0.1	0.6±0.1^{b}	0.23±0.05	0.09±0.01	7.3±0.4	2.3±0.1^{b}	0.9±0.1^{b}	0.30±0.04^{b}	0.5±0.1^{b}	4.0±0.3^{b}
	1 kGy	4.5±0.3	1.7±0.2	0.4±0.1^{c}	0.20±0.03	0.10±0.02	7.0±0.5	2.6±0.1^{a}	1.1±0.1^{a}	0.37±0.05^{a}	0.7±0.1^{a}	4.8±0.3^{a}
	10 kGy	4.3±0.3	1.6±0.2	0.9±0.1^{a}	0.21±0.02	0.09±0.02	7.2±0.4	2.6±0.2^{a}	1.0±0.1^{a}	0.38±0.04^{a}	0.6±0.1^{a}	4.6±0.3^{a}
p-values	Homoscedasticity[2]	0.993	0.685	0.202	0.086	0.510	0.514	0.557	0.737	0.700	0.526	0.987
	Normal distribution[3]	0.331	0.445	0.069	0.711	0.044	0.747	0.587	0.657	0.404	0.574	0.800
	1-way ANOVA[4]	0.157	0.105	<0.001	0.102	0.337	0.198	<0.001	0.002	0.001	<0.001	<0.001

[1]The results are presented as the mean±SD. [2]Homoscedasticity among irradiation doses was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test. [4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one irradiation dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

Table 3. Fatty acids profile (relative percentage) of *Arenaria montana* submitted do different doses of electron-beam or gamma irradiation.[1]

	E-beam irradiation			*p*-values			Gamma irradiation			*p*-values		
	0 kGy	1 kGy	10 kGy	Homoscedasticity[2]	Normal distribution[3]	1-way ANOVA[4]	0 kGy	1 kGy	10 kGy	Homoscedasticity[2]	Normal distribution[3]	1-way ANOVA[4]
C12:0	0.9±0.1^{b}	0.7±0.1^{c}	1.0±0.1^{a}	0.050	0.435	<0.001	1.0±0.1^{b}	1.3±0.1^{a}	1.0±0.1^{b}	0.219	0.809	<0.001
C14:0	1.4±0.1^{b}	1.3±0.1^{b}	2.8±0.2^{a}	0.496	<0.001	<0.001	1.9±0.1^{a}	1.5±0.1^{b}	1.6±0.2^{b}	0.636	0.661	<0.001
C15:0	0.9±0.1^{b}	0.8±0.1^{b}	1.0±0.1^{a}	0.740	0.142	<0.001	1.0±0.1^{a}	0.7±0.1^{b}	0.8±0.1^{b}	0.680	0.101	<0.001
C16:0	26±1^{a}	24±1^{b}	23±1^{b}	0.410	0.344	<0.001	22±1^{b}	23±1^{ab}	24±1^{a}	0.576	0.670	0.001
C17:0	1.1±0.1^{b}	1.3±0.1^{a}	1.3±0.1^{a}	0.316	0.377	<0.001	1.2±0.1^{a}	1.1±0.1^{b}	1.3±0.1^{a}	0.278	0.312	0.001
C18:0	5.0±0.3^{c}	5.7±0.2^{b}	6.6±0.3^{a}	0.210	0.185	<0.001	6.0±0.2^{a}	5.5±0.2^{b}	5.6±0.3^{b}	0.654	0.624	<0.001
C18:1n9	10.1±0.4^{b}	13.4±0.3^{a}	13.2±0.5^{a}	0.563	<0.001	<0.001	12.2±0.3^{a}	11.4±0.2^{b}	10.7±0.3^{c}	0.518	0.287	<0.001
C18:2n6	17.2±0.5^{a}	17.5±0.4^{a}	14.7±0.4^{b}	0.627	0.001	<0.001	14.8±0.4^{b}	16.6±0.3^{a}	16.3±0.4^{a}	0.496	0.011	<0.001
C18:3n6	2.7±0.1^{a}	2.8±0.2^{a}	2.4±0.2^{b}	0.389	0.631	<0.001	2.6±0.2^{b}	3.1±0.2^{a}	2.8±0.3^{b}	0.765	0.593	0.002
C18:3n3	21.6±0.5^{a}	19.6±0.5^{b}	17.4±0.5^{c}	0.998	0.107	<0.001	22.0±0.4^{b}	22.4±0.4^{ab}	22.7±0.3^{a}	0.504	0.331	0.002
C20:0	2.2±0.1^{b}	2.3±0.2^{b}	2.5±0.2^{a}	0.110	0.776	0.001	2.0±0.1^{a}	1.7±0.2^{b}	1.9±0.2^{a}	0.453	0.471	0.001
C20:1	0.5±0.1	0.6±0.1	0.6±0.1	0.004	0.206	0.218	0.6±0.1^{b}	0.8±0.1^{a}	0.6±0.1^{b}	0.579	0.599	<0.001
C20:3n6	1.4±0.1^{a}	1.1±0.1^{b}	1.1±0.1^{b}	0.177	0.335	<0.001	1.5±0.1^{a}	1.4±0.2^{a}	1.2±0.2^{b}	0.466	0.170	0.002
C20:4n6	1.9±0.1^{b}	1.9±0.1^{b}	2.9±0.2^{a}	0.004	<0.001	<0.001	3.6±0.2^{a}	2.6±0.2^{b}	2.4±0.2^{b}	0.740	0.002	<0.001
C20:5n3	0.8±0.1^{b}	0.5±0.1^{c}	1.1±0.1^{a}	0.002	0.008	<0.001	0.7±0.1^{a}	0.6±0.1^{b}	0.5±0.1^{c}	0.089	0.114	<0.001
C22:0	3.2±0.2^{a}	2.9±0.1^{b}	2.9±0.2^{b}	0.038	0.210	<0.001	3.1±0.3^{a}	1.9±0.2^{c}	2.4±0.3^{b}	0.309	0.195	<0.001
C24:0	1.3±0.1^{b}	1.4±0.1^{b}	1.6±0.2^{a}	<0.001	0.020	<0.001	1.7±0.1^{a}	1.2±0.2^{c}	1.4±0.1^{b}	0.121	0.962	<0.001
SFA	42.7±0.5^{b}	41.8±0.5^{c}	45.4±0.5^{a}	0.881	0.017	<0.001	41±1^{a}	39±1^{b}	41±1^{a}	0.358	0.583	0.001
MUFA	10.9±0.3^{b}	14.2±0.2^{a}	14.0±0.5^{a}	0.193	<0.001	<0.001	13.1±0.3^{a}	13.0±0.3^{a}	12.0±0.2^{b}	0.467	0.014	<0.001
PUFA	46.4±0.5^{a}	44.0±0.5^{b}	40.6±0.5^{c}	0.709	0.030	<0.001	46±1^{b}	48±1^{a}	47±1^{ab}	0.107	0.330	0.002

[1]The results are presented as the mean±SD. [2]Homoscedasticity among irradiation doses was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test. [4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one irradiation dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

Table 4. Tocopherols composition (mg/100 g dw) of *Arenaria montana* submitted do different doses of electron-beam or gamma irradiation.[1]

		α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Tocopherols
E-beam irradiation					
Dose	0 kGy	4.6±0.3^{a}	0.26±0.03^{a}	0.29±0.03^{a}	5.2±0.3^{a}
	1 kGy	3.9±0.2^{b}	0.23±0.03^{ab}	0.28±0.03^{ab}	4.4±0.3^{b}
	10 kGy	3.7±0.3^{b}	0.21±0.04^{b}	0.25±0.03^{b}	4.2±0.3^{b}
p-values	Homoscedasticity[2]	0.375	0.374	0.895	0.595
	Normal distribution[3]	0.123	0.138	0.247	0.231
	1-way ANOVA[4]	<0.001	0.040	0.029	<0.001
Gamma irradiation					
Dose	0 kGy	4.8±0.2^{a}	0.28±0.03^{a}	0.16±0.02^{ab}	5.3±0.2^{a}
	1 kGy	4.8±0.1^{a}	0.29±0.03^{a}	0.15±0.01^{b}	5.2±0.2^{a}
	10 kGy	3.5±0.2^{b}	0.20±0.03^{b}	0.19±0.03^{a}	3.8±0.2^{b}
p-values	Homoscedasticity[2]	0.435	0.691	0.005	0.342
	Normal distribution[3]	<0.001	0.526	0.120	<0.001
	1-way ANOVA[4]	<0.001	<0.001	0.004	<0.001

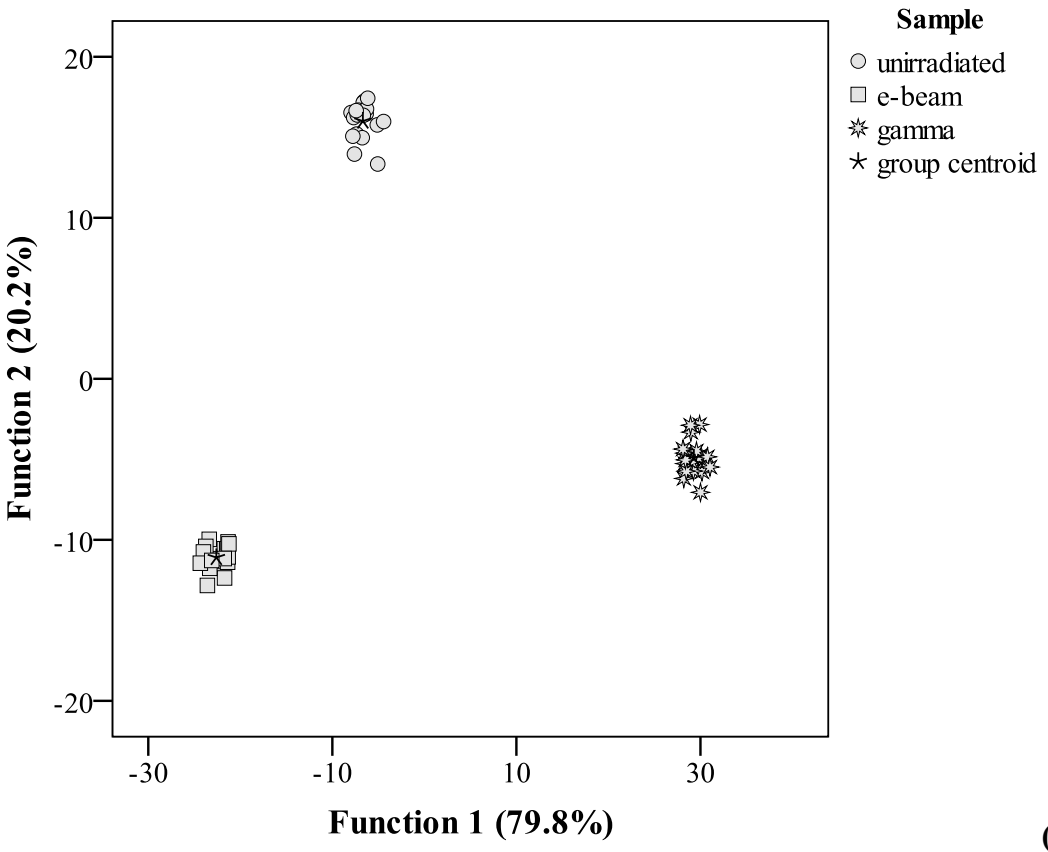
[1]The results are presented as the mean±SD. [2]Homoscedasticity among irradiation doses was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test. [4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one irradiation dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

Table 5. Antioxidant properties of extracts from *Arenaria montana* submitted to electron beam or gamma irradiation (GI).[1] EC_{50} values (μg/mL) are presented for all assays except phenols, expressed as mg GAE/g extract. The results are presented as the mean±SD

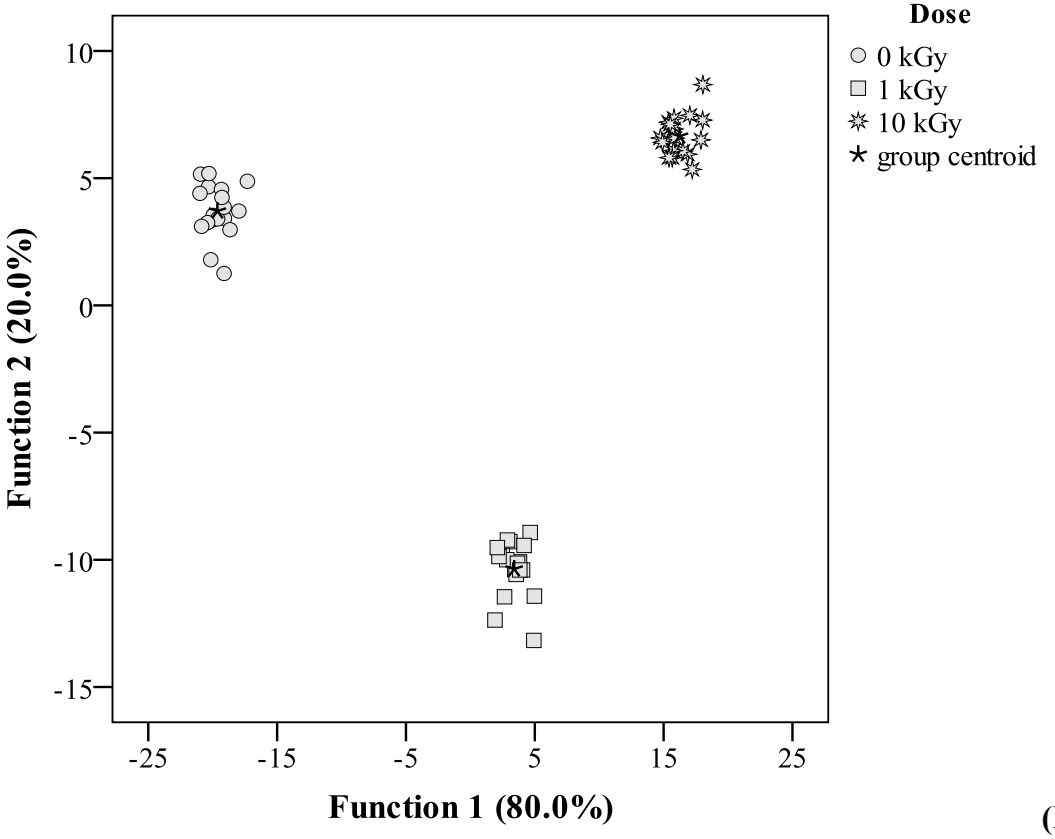
		DPPH scavenging activity		Reducing power		β-carotene bleaching inhibition		TBARS formation inhibition		Phenols	
		Infusion	MeOH	Infusion	MeOH	Infusion	MeOH	Infusion	MeOH	Infusion	MeOH
Electron beam											
	0 kGy	3532±175^{b}	988±20^{a}	1592±15^{c}	528±3^{a}	3658±120^{c}	1772±52^{b}	310±4^{c}	97±2^{a}	40±1^{a}	102±5^{c}
	1 kGy	3998±147^{a}	813±80^{b}	1816±12^{b}	509±4^{b}	4230±227^{b}	1450±131^{c}	365±35^{b}	60±2^{c}	35±1^{b}	109±1^{b}
	10 kGy	3945±338^{a}	631±34^{c}	1954±8^{a}	441±3^{c}	7210±517^{a}	1906±70^{a}	427±29^{a}	72±2^{b}	33±1^{c}	119±1^{a}
	Homoscedasticity[1]	0.011	<0.001	0.198	0.604	<0.001	0.001	0.003	0.684	0.113	<0.001
p-values	Normal distribution[2]	0.750	0.003	0.001	<0.001	<0.001	0.054	0.003	0.001	0.001	0.008
	1-way ANOVA[3]	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Gamma irradiation											
	0 kGy	3475±228^{b}	972±55^{b}	1613±12^{b}	520±8^{a}	2895±173^{b}	665±9^{c}	487±41^{b}	164±3^{c}	37±1^{a}	116±2^{c}
	1 kGy	3619±109^{b}	958±26^{b}	1619±18^{b}	498±13^{b}	4489±325^{a}	875±18^{b}	579±22^{a}	230±2^{b}	37±1^{a}	122±1^{a}
	10 kGy	3942±86^{a}	1962±160^{a}	1709±18^{a}	507±2^{b}	4324±144^{a}	1134±21^{a}	633±107^{a}	245±3^{a}	35±1^{b}	119±1^{b}
	Homoscedasticity[1]	<0.001	<0.001	0.420	0.003	0.052	0.012	0.003	0.013	<0.001	0.001
p-values	Normal distribution[2]	0.043	<0.001	0.002	0.102	0.001	0.001	0.027	<0.001	0.008	0.014
	1-way ANOVA[3]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

MeOH- Methanol; GAE- Gallic acid equivalents; [1]Homoscedasticity among irradiation doses was tested by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$. [2]Normal distribution of the residuals was evaluated using Shapiro-Wilk test. [3]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one dose differs from the others. For each species, means within a column with different letters differ significantly ($p<0.05$).

Figure 1.


Sample
unirradiated
e-beam
gamma
group centroid
Function 2 (20.2%)
Function 1 (79.8%)
20
10
0
-10
-20
-30
-10
10
30

(A)


Dose
0 kGy
1 kGy
10 kGy
group centroid
Function 2 (20.0%)
Function 1 (80.0%)
10
5
0
-5
-10
-15
-25
-15
-5
5
15
25

(B)